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Entitled: **Rapid Detection And
Identification Of Pathogens**

INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 CFR § 1.8(a)(1)(i)(A)

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By:

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Sir or Madam:

The citations listed below, copies attached, may be material to the examination of the above-identified application, and are therefore submitted in compliance with the duty of disclosure defined in 37 C.F.R. §§ 1.56 and 1.97. The Examiner is requested to make these citations of official record in this application.

The following patents are referred to in the body of the specification.

- U.S. Patent No. 4,683,195 issued to Mullis;
- U.S. Patent No. 4,683,202 issued to Mullis *et al.*;
- U.S. Patent No. 5,108,892 issued to Burke *et al.*;
- U.S. Patent No. 5,144,019 issued to Rossi *et al.*;
- U.S. Patent No. 4,511,502 issued to Builder & Ogez;
- U.S. Patent No. 4,518,526 issued to Olson;

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- U.S. Patent No. 4,511,503 issued to Olson & Pai;
- U.S. Patent No. 4,512,922 issued to Jones *et al.*;
- PCT Application No. WO 90/01069 A1 to Segev;
- PCT Application No. WO 92/06200 to Gelfand *et al.*;
- PCT Application No. WO 91/09950 to Gelfand *et al.*;
- PCT Publication WO 90/15157 to Lane *et al.*.

The following printed publications are referred to in the body of the specification:

- Barany, "Genetic disease detection and DNA amplification using cloned thermostable ligase," Proc. Natl. Acad. Sci., 88:189 (1991);
- Barany, "The Ligase Chain Reaction in a PCR World," PCR Methods and Applic., 1:5 (1991);
- Wu and Wallace, "The Ligation Amplification Reaction (LAR) - Amplification of Specific DNA Sequences Using Sequential Rounds of Template-Dependent Ligation," Genomics 4:560 (1989);
- Guatelli *et al.*, "Isothermal, *in vitro* amplification of nucleic acids by a multienzyme reaction modeled after retroviral replication," Proc. Natl. Acad. Sci., 87:1874-1878 (1990) with an erratum at Proc. Natl. Acad. Sci., 87:7797 (1990);
- Kwoh *et al.*, "Transcription-based amplification system and detection of amplified human immunodeficiency virus type 1 with a bead-based sandwich hybridization format," Proc. Natl. Acad. Sci., 86:1173-1177 (1989);
- Fahy *et al.*, "Self-sustained Sequence Replication (3SR): An Isothermal Transcription-based Amplification System Alternative to PCR," PCR Meth. Appl., 1:25-33 (1991);
- Landgren, "Molecular mechanics of nucleic acid sequence amplification," Trends in Genetics 9:199 (1993);

- Mullis, "The Polymerase Chain Reaction in an Anemic Mode: How to Avoid Cold Oligodeoxyribonuclear Fusion," PCR Methods Applic., 1:1 (1991);
- Kwok *et al.*, "Effects of primer-template mismatches on the polymerase chain reaction: Human immunodeficiency virus type 1 model studies," Nucl. Acids Res., 18:999 (1990);
- Duck *et al.*, "Probe Amplifier System Based on Chimeric Cycling Oligonucleotides," BioTech., 9:142 (1990);
- Urdea *et al.*, "A novel method for the rapid detection of specific nucleotide sequences in crude biological samples without blotting or radioactivity; application to the analysis of hepatitis B virus in human serum," Gene 61:253-264 (1987);
- Gogos *et al.*, "Detection of single base mismatches of thymine and cytosine residues by potassium permanganate and hydroxylamine in the presence of tetralkylammonium salts," Nucl. Acids Res., 18:6807-6817 (1990);
- Barlow and Lehrach, "Genetics by gel electrophoresis: the impact of pulsed field gel electrophoresis on mammalian genetics," Trends Genet., 3:167 (1987);
- Perlman and Butow, "Mobile Introns and Intron-Encoded Proteins," Science 246:1106 (1989);
- Conner, *et al.*, "Detection of sickle cell β^S -globin allele by hybridization with synthetic oligonucleotides," Proc. Natl. Acad. Sci. 80:278-282 (1983);
- Vogelstein *et al.*, "Genetic Alterations During Colorectal-Tumor Development," N. Eng. J. Med. 319:525-532 (1988);
- Farr *et al.*, "Analysis of *RAS* gene mutations in acute myeloid leukemia by polymerase chain reaction and oligonucleotide probes," Proc. Natl. Acad. Sci. 85:1629-1633 (1988);
- Lyons, *et al.*, "Two G Protein Oncogenes in Human Endocrine Tumors," Science 249:655-659 (1990);

- Abrams *et al.*, "Comprehensive Detection of Single Base Changes in Human Genomic DNA Using Denaturing Gradient Gel Electrophoresis and a GC Clamp," *Genomics* 7:463-475 (1990);
- Sheffield, *et al.*, "Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes," *Proc. Natl. Acad. Sci.*, 86:232-236 (1989);
- Lerman and Silverstein, "Computational Simulation of DNA Melting and Its Application to Denaturing Gradient Gel Electrophoresis," *Meth. Enzymol.*, 155:482-501 (1987);
- Wartell *et al.*, "Detecting base pair substitutions in DNA fragments by temperature-gradient gel electrophoresis," *Nucl. Acids Res.*, 18:2699-2701 (1990);
- Smith *et al.*, "Novel Method of Detecting Single Base Substitutions in RNA Molecules by Differential Melting Behavior in Solution," *Genomics* 3:217-223 (1988);
- Borresen *et al.*, "Constant denaturant gel electrophoresis as a rapid screening technique for p53 mutations," *Proc. Natl. Acad. Sci. USA* 88:8405 (1991);
- Scholz, *et al.*, "Rapid screening for Tp53 mutations by temperature gradient gel electrophoresis: a comparison with SSCP analysis," *Hum. Mol. Genet.* 2:2155 (1993);
- Hayashi, "PCR-SSCP: A Simple and Sensitive Method for Detection of Mutations in the Genomic DNA," *PCR Meth. Appl.*, 1:34-38, (1991);
- Orita, *et al.*, "Rapid and Sensitive Detection of Point Mutations and DNA Polymorphisms Using the polymerase Chain Reaction," *Genomics* 5:874-879, (1989);
- Liu and Sommer, "Parameters Affecting the Sensitivity of Dideoxy Fingerprinting and SSCP," *PCR Methods Appl.*, 4:97 (1994);

- Marmur and Lane, "Strand Separation and Specific Recombination in Deoxyribonucleic acids: Biological Studies," Proc. Natl. Acad. Sci. USA 46:453 (1960);
- Doty *et al.*, "Strand Separation and Specific Recombination in Deoxyribonucleic Acids: Physical Chemical Studies," Proc. Natl. Acad. Sci. USA 46:461 (1960);
- Wallace *et al.*, "Application of synthetic oligonucleotides to the diagnosis of human genetic diseases," Biochimie 67:755 (1985);
- Studencki and Wallace, "Allele-Specific Hybridization using Oligonucleotide Probes of Very High Specific Activity: Discrimination of the Human β^A - and β^S -Globin Genes," DNA 3:1 (1984);
- Studencki *et al.*, "Discrimination among the Human β^A , β^S , and β^C -Globin Genes Using Allele-Specific Oligonucleotide Hybridization Probes," Human Genetics 37:42 (1985);
- Harrington and Liener, "Functional domains within FEN-1 and RAD2 define a family of structure-specific endonucleases: implications for nucleotide excision repair," Genes and Develop. 8:1344 (1994);
- Murante *et al.*, "The Calf 5'- to 3'-Exonuclease Is Also an Endonuclease with Both Activities Dependent on Primers Annealed Upstream of the Point of Cleavage," J. Biol. Chem. 269:1191 (1994);
- Kornberg, *DNA Replication*, W.H. Freeman and Co., San Francisco, pp. 127-139 (1980);
- Tindall and Kunkell, "Fidelity of DNA Synthesis by the *Thermus aquaticus* DNA Polymerase," Biochem. 27:6008 (1988);
- Brutlag *et al.*, "An Active Fragment of DNA Polymerase Produced By Proteolytic Cleavage," Biochem. Biophys. Res. Commun. 37:982 (1969);
- Erlich *et al.*, "Recent Advances in the Polymerase Chain Reaction," Science 252:1643 (1991);

- Setlow and Kornberg, "Deoxyribonucleic Acid Polymerase: Two Distinct Enzymes in One Polypeptide," J. Biol. Chem. 247:232 (1972);
- Gelfand, PCR Technology - Principles and Applications for DNA Amplification (H.A. Erlich, Ed.), Stockton Press, New York, p. 19 (1989);
- Holland *et al.*, "Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of *Thermus aquaticus* DNA polymerase," Proc. Natl. Acad. Sci. USA 88:7276 (1991);
- Lawyer *et al.*, "Isolation, Characterization, and Expression in *Escherichia coli* of the DNA Polymerase Gene from *Thermus aquaticus*," J. Biol. Chem. 264:6427 (1989);
- Akhmetzjanov and Vakhitov, "Molecular cloning and nucleotide sequence of the DNA polymerase gene from *Thermus flavus*," Nucl. Acids Res. 20:5839 (1992);
- Setlow *et al.*, "Deoxyribonucleic Acid Polymerase: Two Distinct Enzymes in One Polypeptide," J. Biol. Chem. 247:224 (1972)
- Levine, "The Tumor Suppressor Genes," Annu. Rev. Biochem. 62:623 (1993);
- Lane and Benchimol, "p53: oncogene or anti-oncogene," Genes Dev. 4:1 (1990);
- Lowe *et al.*, "p53-Dependent Apoptosis Modulates the Cytotoxicity of Anticancer Agents," Cell 74:957 (1995);
- Hollstein, *et al.*, "Database of p53 gene somatic mutations in human tumors and cell lines," Nucleic Acids Res. 22:3551 (1994);
- Cariello *et al.*, "Database and software for the analysis of mutations at the human p53 gene," Nucleic Acids Res. 22:3549 (1994);
- Hollstein *et al.*, "Database of p53 gene somatic mutations in human tumors and cell lines," Nucleic Acids Res. 22:3551 (1994);

- Higuchi, R., In Ehrlich, H.A. (Ed.), PCR Technology: Principles and Applications for DNA Amplification, Stockton Press, New York, pp. 61-70 (1991);
- Nelson and Long, "A General Method of Site-Specific Mutagenesis Using a Modification of the *Thermus aquaticus* Polymerase Chain Reaction," *Analytical Biochem.* 180:147 (1989);
- Altamirano *et al.*, "Identification of Hepatitis C Virus Genotypes among Hospitalized Patients in British Columbia, Canada," *J. Infect. Dis.* 171:1034 (1995);
- Kanai *et al.*, "HCV genotypes in chronic hepatitis C and response to interferon," *Lancet* 339:1543 (1992);
- Yoshioka *et al.*, "Detection of Hepatitis C Virus by Polymerase Chain Reaction and Response to Interferon- α Therapy: Relationship to Genotypes of Hepatitis C Virus," *Hepatology* 16:293 (1992);
- Okamoto *et al.*, "Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources," *J. Gen. Virol.* 73:673 (1992);
- Frieden *et al.*, "The Emergence of Drug-Resistant Tuberculosis in New York City," *New Engl. J. Med.* 328:521 (1993);
- Hughes, *Scrip Magazine* May (1994);
- Jacobs, Jr., "Multiple-Drug-Resistant Tuberculosis," *Clin. Infect. Dis.* 19:1 (1994);
- Donnabella *et al.*, "Isolation of the Gene for the β Subunit of RNA Polymerase from Rifampicin-resistant *Mycobacterium tuberculosis* and Identification of New Mutations," *Am. J. Respir. Dis.* 11:639 (1994);
- Jacobs, Jr. *et al.*, "Rapid Assessment of Drug Susceptibilities of *Mycobacterium tuberculosis* by Means of Luciferase Reporter Phages," *Science* 260:819 (1993)

- Shinnick and Jones in *Tuberculosis: Pathogenesis, Protection and Control*, Bloom, ed., American Society of Microbiology, Washington, D.C. (1994), pp. 517-530;
- Yule, "Amplification-Based Diagnostics Target TB," *Bio/Technology* 12:1335 (1994);
- Heym *et al.*, "Implications of multidrug resistance for the future of short-course chemotherapy of tuberculosis: a molecular study," *Lancet* 344:293 (1994);
- Morris *et al.*, "Molecular Mechanisms of Multiple Drug Resistance in Clinical Isolates of *Mycobacterium tuberculosis*," *J. Infect. Dis.* 171:954 (1995);
- Banerjee *et al.*, "*inhA*, a Gene Encoding a Target for Isoniazid and Ethionamide in *Mycobacterium tuberculosis*," *Science* 263:227 (1994);
- Woese, *Bacterial Evolution. Microbiological Reviews*, vol 51, No. 2. (1987);
- Shibata, "Preparation of Nucleic Acids for Archival Material," in *PCR: The Polymerase Chain Reaction*, Mullis *et al.*, eds., Birkhauser, Boston, pp. 47-54 (1994);
- Saiki *et al.*, "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase," *Science* 239:487 (1988);
- Mullis and Faloona, "Specific Synthesis of DNA *in Vitro* via a Polymerase-Catalyzed Chain Reaction," *Methods in Enzymology* 155:335 (1987);
- M. Bargseid *et al.*, "A High Fidelity Thermostable DNA Polymerase Isolated from *Pyrococcus furiosus*," *Strategies* (Startagene, LaJolla, CA) 4:34 (1991);
- Perler *et al.*, "Intervening sequences in an Archaea DNA polymerase gene," *Proc. Natl. Acad. Sci. USA* 89:5577 (1992);
- Kaledin *et al.*, "Isolation and Properties of DNA Polymerase From the Extremely Thermophilic Bacterium *Thermus flavus*," *Biokhimiya* 46:1576 (1981);

- Carballeira *et al.*, "Purification of a Thermostable DNA Polymerase from *Thermus thermophilus* HB8, Useful in the Polymerase Chain Reaction," *Biotechniques* 9:276 (1990);
- Myers *et al.*, "Reverse Transcription and DNA amplification by a *Thermus thermophilus* DNA Polymerase," *Biochem.* 30:7661 (1991);
- Ito *et al.*, "Compilation and alignment of DNA polymerase sequences," *Nucl. Acids Res.* 19:4045 (1991);
- Mathur *et al.*, "The DNA polymerase gene from the hyperthermophilic marine archaeobacterium *Pyrococcus furiosus*, shows sequence homology with α -like DNA polymerases," *Nucl. Acids. Res.* 19:6952 (1991);
- Dunn *et al.*, "Complete Nucleotide Sequence of Bacteriophage T7 DNA and the Locations of T7 Genetic Elements," *J. Mol. Biol.* 166:477 (1983);
- Antao *et al.*, "A thermodynamic study of unusually stable RNA and DNA hairpins," *Nucl. Acids Res.* 19:5901 (1991);
- Stark, "Multicopy expression vectors carrying the *lac* repressor gene for regulated high-level expression of genes in *Escherichia coli*," *Gene* 5:255 (1987);
- Studier and Moffatt, "Use of Bacteriophage T7 RNA Polymerase to Direct Selective High-level Expression of Cloned Genes," *J. Mol. Biol.* 189:113 (1986);
- Sambrook *et al.*, Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 1.63-1.69 (1989);
- Engelke, "Purification of *Thermus Aquaticus* DNA Polymerase Expressed in *Escherichia coli*," *Anal. Biochem* 191:396 (1990);
- Copley and Boot, "Exonuclease Cycling Assay: An Amplified Assay for the Detection of Specific DNA Sequences," *BioTechniques* 13:888 (1992);

- King, R.A., *et al.*, "Non-random Distribution of Missense Mutations Within the Human Tyrosinase Gene in Type I (Tyrosinase-related) Oculocutaneous Albinism," *Mol. Biol. Med.* 8:19 (1991);
- Giebel *et al.*, "Organization and Nucleotide Sequences of the Human Tyrosinase Gene and a Truncated Tyrosinase-Related Segment," *Genomics* 9:435 (1991);
- Spritz, "Molecular genetics of oculocutaneous albinism," *Human Molecular Genetics* 3:1469 (1994);
- Giebel *et al.*, "A Tyrosinase Gene Missense Mutation in Temperature-sensitive Type I Oculocutaneous Albinism," *J. Clin. Invest.* 87:1119 (1991);
- Bouchard *et al.*, "Induction of Pigmentation in Mouse Fibroblasts by Expression of Human Tyrosinase cDNA," *J. Exp. Med.* 169:2029 (1989);
- Orkin and Kazazian, "The Mutation and Polymorphism of the Human β -Globin Gene and its Surrounding DNA," *Annu. Rev. Genet.* 18:13 (1984);
- Collins and Weissman, "The Molecular Genetics of Human Hemoglobin," *Prog. Nucleic Acid Res. Mol. Biol.* 31:315 (1984);
- Lawn *et al.*, "The Nucleotide Sequence of the Human β -Globin Gene," *Cell* 21:647 (1980);
- Orkin and Goff, "Nonsense and Frameshift Mutations in β^0 -Thalassemia Detected in Cloned β -Globin Genes," *J. Biol. Chem.* 256:9782 (1981);
- Goldsmith *et al.*, "'Silent' nucleotide substitution in a β^+ -thalassemia globin gene activates splice site in coding sequence RNA," *Proc. Natl. Acad. Sci. USA* 80:2318 (1983);
- Giddings *et al.*, "An adaptive, object oriented strategy for base calling in DNA sequence analysis," *Nucl. Acids Res.* 21:4530 (1993);
- Trivedi *et al.*, "Selective Amplification of Simian Immunodeficiency Virus Genotypes after Intrarectal Inoculation of Rhesus Monkeys," *Journal of Virology* 68:7649 (1994);

- Nugent *et al.*, "Characterization of the Apurinic Endonuclease Activity of *Drosophila* Rrp1," *Biochemistry*, 32:11445 (1993);
- Bardwell *et al.*, "Specific Cleavage of Model Recombination and Repair Intermediates by the Yeast Rad1-Rad10 DNA Endonuclease," *Science* 265:2082 (1994);
- Orkin *et al.*, "Abnormal RNA processing due to the exon mutation of β^E -globin gene," *Nature*, 300:768 (1982);
- Spritz *et al.*, "Base substitution in an intervening sequence of a β^+ -thalassemic human globin gene," *Proc. Natl. Acad. Sci. USA*, 78:2455 (1981);
- Baker *et al.*, "Suppression of Human Colorectal Carcinoma Cell Growth by Wild-Type p53," *Science* 249:912 (1990);
- Chen *et al.*, "Genetic Mechanisms of Tumor Suppression by the Human p53 Gene," *Science* 250:1576 (1990);
- Hollstein *et al.*, "p53 Mutations in Human Cancers," *Science* 253:49 (1991);
- Caron de Fromental and Soussi, "TP53 Tumor Suppressor Gene: A Model for Investigating Human Mutagenesis," *Genes, Chromosomes and Cancer* 4:1 (1992).
- Inchauspe *et al.*, "Use of Conserved Sequences from Hepatitis C Virus for the Detection of Viral RNA in Infected Sera by Polymerase Chain Reaction," *Hepatology* 14:595 (1991);
- Miller *et al.*, "The *rpoB* Gene of *Mycobacterium tuberculosis*," *Antimicrob. Agents Chemother.*, 38:805 (1994);
- Cockerill *et al.*, "Rapid Identification of a Point Mutation of the *Mycobacterium tuberculosis* Catalase-Peroxidase (*katG*) Gene Associated with Isoniazid Resistance," *J. Infect. Dis.* 171:240 (1995);
- Greisen *et al.*, "PCR Primers and Probes for the 16S rRNA Gene of Most Species of Pathogenic Bacteria, Including Bacteria Found in Cerebrospinal Fluid," *J. Clin. Microbiol.* 32:335 (1994);

- Widjoatmondjo *et al.*, "Rapid Identification of Bacteria by PCR-Single-Strand Conformation Polymorphism," J. Clin. Microbiol. 32:3002 (1994);
- Maidak *et al.*, "The Ribosomal Database project," Nucleic Acids Res., 22:3485 (1994);
- McConlogue *et al.*, "Structure-independent DNA amplification by PCR using 7-deaza-2'-deoxyguanosine," Nucleic Acids Res. 16:20 (1988);

Applicants have become aware of the following printed publications which they believe may be material to the examination of the instant application:

- U.S. Patent No. 5,422,253 to Dahlberg *et al.* describes methods for the cleavage of a single-stranded target nucleic acid molecule by forming a cleavage structure comprising the target nucleic acid strand and a pilot nucleic acid (*i.e.*, an oligonucleotide). Cleavage of nucleic acids at regions of intramolecular annealing in the absence of pilot oligonucleotides is also described. In contrast to the claimed invention, this reference does not describe the cleavage of nucleic acid substrates derived from microorganisms to produce a characteristic set of cleavage products.
- U.S. Patent No. 5,541,311 to Dahlberg *et al.* describes the generation of DNA sequences encoding modified DNA polymerases that retain 5' nuclease activity but lack synthetic activity and the use of these modified DNA polymerases for the detection of specific nucleic acid sequences in an assay employing cleavage structures formed by annealing a target nucleic acid with one or more oligonucleotides. In contrast to the claimed invention, this reference does not describe the cleavage of nucleic acid substrates derived from microorganisms to produce a characteristic set of cleavage products;

- U.S. Patent No. 5,614,402 to Dahlberg *et al.* describes methods for the detection of specific nucleic acid sequences in an assay employing modified DNA polymerases lacking synthetic activity and cleavage structures formed by annealing a target nucleic acid with one or more oligonucleotides. In contrast to the claimed invention, this reference does not describe the cleavage of nucleic acid substrates derived from microorganisms to produce a characteristic set of cleavage products;
- D.S. Sigman *et al.*, "Chemical Nucleases," *Chemical Reviews* 93:2295-2316 (1993) describe the use of chemical (*i.e.*, non-enzymatic) nucleases for the cleavage of nucleic acids. In contrast, the claimed invention specifies that an enzymatic cleavage means is used to cleave a nucleic acid substrate containing cleavage structures (*i.e.*, single strands of nucleic acids containing a region(s) of secondary structure). Sigman *et al.* fail to teach the enzymatic cleavage of nucleic acid substrates containing cleavage structures;
- T.R. Cech *et al.*, "Secondary Structure of the *Tetrahymena* ribosomal RNA intervening sequence, Structural homology with fungal mitochondrial intervening sequences," *Proc. Natl. Acad. Sci. USA* 80:3903 (1983) report the cleavage of a single wild-type RNA substrate (the *Tetrahymena* IVS RNA) using nucleases specific for the cleavage of RNA substrates (*i.e.*, RNases). Cech *et al.* do not teach the cleavage of a nucleic acid substrate followed by the comparison of the resulting cleavage products with a reference control nor the formation of cleavage structures by heating and cooling of a nucleic acid substrate nor the extraction of nucleic acids from a sample followed by cleavage of cleavage structures. Further, Cech *et al.* do not teach the use of nucleases in a solution containing manganese;

- C.R. Woese *et al.*, "Detailed Analysis of the Higher Order Structure of 16S Like Ribosomal Ribonucleic Acids," *Microbiology Reviews* 47:621 (1983) provides a review summarizing evidence for supporting proposed secondary structures for 16S-like ribosomal RNAs. Woese *et al.* teach that the primary sequences of corresponding rRNAs are compared in order to confirm the predicted secondary structures of those rRNAs. In contrast to the claimed invention, Woese *et al.* do not teach the cleavage of a nucleic acid substrate containing cleavage structures to generate a characteristic set of cleavage products. Woese *et al.* further do not teach the cleavage of cleavage structures using 5' nucleases derived from DNA polymerases nor the formation of cleavage structures by heating and cooling of a nucleic acid substrate;
- Hoheisel *et al.*, "On The Activities of *Escherichia coli* Exonuclease III," *Anal. Biochem.* 209:238-246 (1993). Hoheisel *et al.* disclose the results of a study of the exonuclease activity of the *E. coli* exonuclease III enzyme. Double stranded nucleic acid sequences which contain an overhang at the 3'-end of up to 4 nt long are cleaved by the enzyme. The disclosure by Hoheisel *et al.* is distinguished from the claimed invention in that Hoheisel *et al.* does not disclose treating the nucleic acid substrate under conditions such that single stranded nucleic acid sequences form a secondary structure(s). Rather, the single stranded overhangs disclosed by Hoheisel *et al.* are cleaved in the absence of the formation of a secondary structure;
- R. Youil *et al.*, "Screening for Mutations by Enzyme Mismatch Cleavage with T4 Endonuclease VII," *Proc. Natl. Acad. Sci. USA* (1995). This paper discloses a method for screening for mutations which utilizes a T4 resolvase enzyme to cleave mismatched heteroduplexes formed between wild-type and mutant DNA. This paper shows that cleavage of double-stranded DNA templates containing a single base mismatch by T4 endonuclease VII is influenced by DNA sequence (*i.e.*, the enzyme is not a structure-specific,

sequence-independent cleavage agent; in contrast, the enzymes used in the methods of the present invention are structure-specific, sequence-independent cleavage agents). The method requires the use of a double-stranded DNA template derived from two different sources of DNA (e.g., a wild-type and mutant form of a gene). In contrast, the methods of the present invention do not require the use of a double-stranded DNA template derived from two different sources of DNA for the detection of mutations;

- European Patent Application No. 912202731.5 (Publication No. 0 482 714 A1) to Sullivan. Sullivan describes a *Thermus aquaticus* gene which encodes a thermostable DNA polymerase (*Taq*) with an altered N-terminus. This alteration is designed to provide improved expression of the *Taq* gene in *Escherichia coli*. In contrast to the present invention, Sullivan does not teach the cleavage of a nucleic acid substrate containing cleavage structures to generate a characteristic set of cleavage products;
- Murphy *et al.*, "Use of the 5' Noncoding Region for Genotyping Hepatitis C Virus," J. Infect. Diseases 169:473 (1994). Murphy *et al.* disclose identification of six hepatitis C virus (HCV) genotypes using restriction length fragment polymorphism (RFLP) of the 5' noncoding region of the HCV genome. The PCR-amplified 5' noncoding region sequences are digested with restriction endonucleases, and the restriction reaction products are separated by gel electrophoresis. Murphy *et al.* report that each of the six HCV genotypes exhibits a unique pattern of restriction cleavage products. The RFLP method of Murphy *et al.* is distinguished from the claimed method in that RFLP relies on the unique locations of restriction enzyme recognition sequences in the 5' noncoding region of the genome of different organisms. This is in contrast to the claimed method which relies on the formation of secondary structures and cleavage of those structures. The RFLP method of Murphy *et al.* does not

comprise either formation or cleavage of secondary structures in the nucleic acid substrate;

- Takada *et al.*, "HCV genotypes in different countries," Lancet 339:808 (1992). Takada *et al.* disclose the prevalence of different HCV genotypes in different countries. HCV-RNA encoding a portion of the NS5 domain of the HCV genome is amplified using reverse transcription polymerase chain reaction. The amplified cDNA fragments are genotyped by slot-blot hybridization with probes specific to each type of HCV or by restriction fragment length polymorphism analysis. The slot-blot method of Takada *et al.* is distinguished from the claimed method because the slot-blot method relies on binding of labelled oligonucleotide sequences to specific nucleotide sequences on the amplified cDNA fragments. In contrast, the claimed method relies on the formation of secondary nucleic acid structures followed by cleavage of those structures. The restriction fragment length polymorphism method of Takada *et al.* also differs from the claimed method. Restriction fragment length polymorphism relies on cleavage at sequence-specific restriction enzyme sites, rather than on cleavage of secondary structures, in the nucleic acid sequence;
- Belkum, "DNA Fingerprinting of Medically Important Microorganisms by Use of PCR," Clin. Microbiol. Rev. 7(2): 174-184 (1994). Belkum discloses the use of PCR fingerprinting for genetic typing of eukaryotic and prokaryotic microorganisms. Nucleic acid sequences are amplified using oligonucleotide primers which target repeat sequences, random sequences, or sequences occurring at multiple sites in the genome. The amplified nucleic acid products are separated by gel electrophoresis and banding pattern homologies are evaluated. The PCR fingerprinting method of Belkum is distinguished from the claimed method in that the PCR fingerprinting method relies on the location and distribution of repeat sequences, random sequences or sequences occurring at multiple sites in the nucleic acid substrate. In contrast, the claimed method

exploits the formation of secondary structures in the nucleic acid substrate.

Additionally, whereas the claimed method comprises cleavage of the secondary nucleic acid structures, PCR fingerprinting does not comprise a cleavage step;

- Wilson *et al.*, "Amplification of Bacterial 16S Ribosomal DNA with Polymerase Chain Reaction," J. Clin. Microbiol. 28(9):1942-1946 (1990).
Wilson *et al.* disclose a method for the detection of eubacteria using PCR amplification of the 16S rDNA. The 16S rDNA sequence is amplified using primers which are highly conserved among eubacteria and which are absent from eucaryotes, archaeobacteria, or mitochondria. The PCR reaction products are separated by gel electrophoresis and the amplified sequences detected. Unlike the claimed method, the PCR method of Wilson *et al.* relies on the relative positions of certain conserved sequences with respect to each other, rather than on formation of secondary structures in the nucleic acid substrate. Moreover, in contrast to the claimed method in which the formed secondary structure is cleaved, the PCR method of Wilson *et al.* does not comprise such a cleavage step;
- Bingen *et al.*, "Use of Ribotyping in Epidemiological Surveillance of Nosocomial Outbreaks," Clin. Microbiol. Rev. 7(3):311-327 (1994). Bingen *et al.* disclose the use of ribotyping in combination with restriction fragment length polymorphism (RFLP) to differentiate microbial isolates. The RFLP method disclosed by Bingen *et al.* is essentially similar to the method used by Takada *et al.*, described *supra*. Both the Takada *et al.* method and the method used by Bingen *et al.* use restriction endonucleases to cleave the substrate DNA. However, instead of using DNA probes, Bingen *et al.* use an rRNA probe for the highly conserved bacterial rRNA genes (*rrn*) to detect the restriction fragments. Unlike the claimed method, the RFLP method of Bingen *et al.* relies on cleavage of the substrate nucleic acid sequence at restriction

enzyme sites, rather than on formation and cleavage of secondary structures in the nucleic acid substrate;

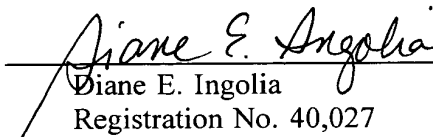
- U.S. Patent No. 5,455,170 to Abramson *et al.* provides DNA sequences encoding the wild-type and mutant forms of a nucleic acid polymerase from *Thermus species* Z05. This reference notes that the reverse transcriptase activity of the *Thermus species* Z05 polymerase has increased activity in the presence of Mn^{2+} as compared to Mg^{2+} . In contrast to the claimed invention, this reference does not teach a method for the cleavage of nucleic acids containing secondary structures. This reference is silent with regard to a teaching that the 5' nuclease activity of a DNA polymerase may function when Mn^{2+} is used in place of Mg^{2+} . Rather this reference speaks of the effect of Mn^{2+} upon the polymerization (*i.e.*, DNA synthetic) activity of a polymerase as measured by cDNA yields during reverse transcription; and
- Tabor *et al.*, "Effect Of Manganese Ions On The Incorporation Of Dideoxynucleotides By Bacteriophage T7 DNA Polymerase And *Escherichia coli* DNA Polymerase I," *Proc. Natl. Acad. Sci. USA* 86:4076-4080 (1989). This reference teaches that the efficiency of incorporation of dideoxynucleotides (*i.e.*, DNA synthetic activity) by T7 DNA polymerase and *E. coli* DNA polymerase I is more efficient when Mn^{2+} is used in place of Mg^{2+} in the reaction buffer. This reference is silent with regard to a teaching that the 5' nuclease activity of a DNA polymerase may function when Mn^{2+} is used in place of Mg^{2+} . In contrast to the claimed invention, this reference does not teach a method for the cleavage of nucleic acids containing secondary structures.

PATENT

Attorney Docket No. **FORS-01756**

This Information Disclosure Statement under 37 C.F.R. §§ 1.56 and 1.97 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that any one or more of these citations constitutes prior art.

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